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*Investigations dealing with the Phenomena of "Clot" Formations.*

Part IV.—*The Diphasic\* Erosive Action of Salts on the Cholate Gel.*

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INTRODUCTION.

In the last communication† of this series, attention was called to the fact that the cholate gel is eroded when immersed in solutions of sodium chloride and other chlorides, and that this erosive action can be "antagonised" by addition to the chloride solutions of relatively small amounts of calcium chloride. The analogy between this action and the biological "antagonistic" action of calcium towards sodium and other salts was also commented upon and illustrated by examples from the researches of Loeb, Osterhout and other investigators.

In addition, however, to the antagonism between calcium salts on the one hand and sodium, potassium and magnesium salts on the other hand, Loeb has shown in the case of *Fundulus* that there is evidence of antagonism between potassium and sodium salts, that is to say that the toxic action of one salt can be rendered more or less innocuous by the addition of the other. Osterhout has shown that certain marine plants will retain their vital

\* The term "diphasic" used in the sub-title to this paper refers to the diphasic form of the curve produced when the concentrations of the eroding salts are plotted as abscissæ and the amounts of erosion as ordinates.

† Schryver, 'Roy. Soc. Proc.,' B, vol. 89, p. 176 (1916).

activities for a longer period in a solution which contains, in addition to sodium and calcium salts, also potassium and magnesium salts, and has formulated the conception of "balanced" salt solutions as necessities for the maintenance of the maximal activities of plants.

The investigations on the salt actions already published have, so far, revealed only the analogy between biological antagonism of calcium to certain other salts, and an antagonistic action of a similar nature on the cholate gel. In view of the conception advanced by one of the authors of this paper, that the protoplasm or cell-membrane contains a gel-forming substance alike in many of its physical properties to cholate gels, it was of interest to determine whether further analogies exist between the biological action of salts and their erosive action on the cholate gel—to ascertain, for example, whether mixtures other than those of calcium chloride and one of the chlorides of alkali metals could form "balanced" solutions in which the gel can maintain its stability.

Now as the hypothesis has been put forward that plant and animal cells contain gel-like structures, and that their normal activities are intimately connected with the maintenance of a certain state of aggregation of such structures, it was of importance to study the action of salts on gels which might simulate as closely as possible those postulated for in the living cell. These should contain, in addition to the substance to which the gel structure is primarily due, certain salts and other bodies to which must be ascribed the relatively high osmotic pressure of the cell. Now in all the experiments already carried out on the erosion of the cholate gel, the material employed has been relatively poor in inorganic salts; the solution from which the gel was prepared by heating contained, in fact, 2 per cent. sodium cholate (the concentration of which was less than N/20) and calcium chloride in the concentration of N/40 (M/80). In the present communication are contained the results of a series of experiments on the action of salts on gels containing larger amounts of added salts. The results indicate that gel stability in the presence of salts is determined by the action of several factors. Much research remains to be accomplished before the laws governing gel formation and disaggregation can be elucidated and the biological actions of salts explained.

#### EXPERIMENTAL.

The experimental method employed was the same as that described in the last communication.\*

\* *Loc. cit.*, p. 176.

Four gels were prepared from the following series of mixtures:—

2 c.c. 4-per-cent. sodium cholate + 0.5 c.c. 3 N/10 CaCl <sub>2</sub> + 1.5 c.c. N/3 LiCl.									
"	"	"	"	"	"	1.5 c.c. N/3 NaCl.			
"	"	"	"	"	"	1.5 c.c. N/3 KCl.			
"	"	"	"	"	"	1.5 c.c. N/3 MgCl <sub>2</sub> .			

The concentration of calcium chloride in the mixtures, therefore, was 3N/80 and of the added salts N/8. The mixtures were introduced into U-tubes of about 3 mm. internal diameter, and clotted by heating for  $\frac{1}{2}$  hour in a thermostat at 50°. The tubes were then removed from the thermostat, and cut into lengths of 75 mm., which were introduced into a series of Petri dishes containing 20 c.c. of the different salt solutions of varying concentrations. After 17 hours, the tubes of gel were removed and the amount of erosion was measured. The experiments were carried out at room temperatures. The numbers given in Table I, which represent the amount of erosion in millimetres, are not strictly comparable, as the experiments of each gel were carried out at different times and at slightly different temperatures. All the experiments with each individual gel were, however, carried out at the same time.

Table I.—Erosion of Gels (in millimetres) containing Different Salts (in Concentrations N/8) when Immersed in Eroding Salt Solutions of Varying Concentrations.

Salt added to gel.	Salt in eroding solution.	Concentrations of the salt in eroding solutions.									
		N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
LiCl	LiCl ...	15	39	56	68	46	28	17	20	21	26
	NaCl ...	14	24	38	19	18	25	34	50	70	>75
	KCl ...	16	19	21	17	20	27	38	52	72	>75
	MgCl <sub>2</sub> ...	12	36	31	22	16	22	30	37	42	46
NaCl	LiCl ...	15	43	64	69	69	61	57	26	19	20
	NaCl ...	15	33	53	44	19	19	22	34	48	66
	KCl ...	18	18	32	22	16	14	22	32	43	55
	MgCl <sub>2</sub> ...	11	34	34	34	26	14	20	28	38	38
KCl	LiCl ...	16	45	62	73	72	66	63	51	18	20
	NaCl ...	14	31	52	50	23	18	22	35	48	56
	KCl ...	19	20	24	24	18	19	25	35	50	65
	MgCl <sub>2</sub> ...	10	29	30	35	27	13	20	29	35	40
MgCl <sub>2</sub>	LiCl ...	44	73	75	75	73	58	32	23	23	17
	NaCl ...	30	50	49	30	16	18	20	32	47	62
	KCl ...	35	40	32	20	16	18	23	35	45	64
	MgCl <sub>2</sub> ...	42	50	50	45	18	16	20	32	45	50

The amount of erosion of the various gels in water lay between 12 and 14 mm.

The results of the erosive action of different salts on the gel containing added potassium chloride are shown graphically in fig. 1, where the concentrations of the eroding solutions are plotted as abscissæ and the amounts of erosion as ordinates. The curves obtained with gels containing other added salts do not differ materially from this one.

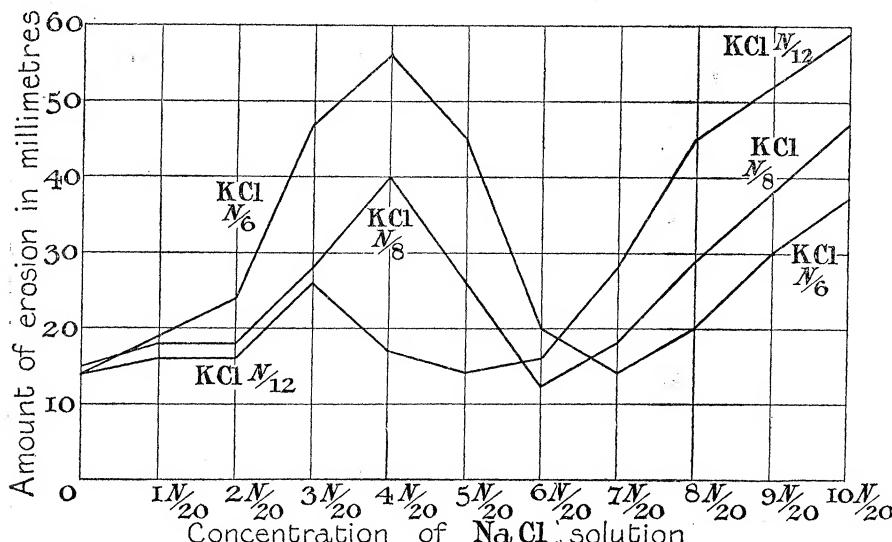


FIG. 1.

It will be noticed that the curves possess a diphasic character, with two minima and two maxima. The amount of erosion increases with increase in the concentration of salt in the eroding solution up to a certain point ( $3N/20$ — $4N/20$ ). On increasing the concentration beyond this, the amount of erosion diminishes, until it reaches a minimum ( $6N/20$  in the case of the chlorides of potassium, magnesium and sodium, and  $9N/20$  in the case of lithium chloride), when it is little more than that which takes place in the presence of pure water. On increasing the concentration above this point the erosion again increases. Results of this character have been obtained constantly; only a portion of them are recorded in this paper.

There is a considerable variation in the effects produced by the various salts in the eroding solution. The maximal eroding effect produced by the lower concentrations, shown both by the amount of erosion and the breadth of the "zone of instability" (as it is convenient to designate the interval between the two minima of the curve), is produced by the lithium salt, followed, in decreasing order of action, by the chlorides of sodium, magnesium and potassium. It has been found by Stiles and Jörgensen\* that the effect

\* 'Annals of Botany,' vol. 29, p. 349 (1915).

of the action of chlorides on the permeability of certain vegetable tissues follows in the same order.

The breadth of the zone of instability is also affected by the concentration of the salts contained within the gel. When the gel is made by heating a solution containing 2-per-cent. sodium cholate and 3N/80 calcium chloride, with no other added salt, the diphasic character of the curve is only marked in the cases where lithium and magnesium chlorides are in the eroding fluid ; in both these cases two maxima in the curve can be observed ; in the cases of sodium and potassium chlorides only faint indications of a diphasic character are obtained.\* The experimental results are given in Table II.

Table II.—Erosive Action (measured in mm.) of Salts on Gel containing no Added Salt. Gel 2 c.c. 4-per-cent. Sodium Cholate + 0.5 c.c. 3N/10  $\text{CaCl}_2$  + 1.5 c.c.  $\text{H}_2\text{O}$ .

	Concentrations of salt solutions.										
	0.	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
LiCl .....	12	13	13	22	20	15	16	23	30	38	60
NaCl .....	—	12	6	12	18	26	42	54	73	>75	>75
KCl .....	—	10	7	9	14	26	35	47	66	67	71
MgCl <sub>2</sub> ...	—	9	19	11	15	27	42	51	52	55	61

Both the breadth of the "zone of instability" and the amount of erosion within this zone can be increased, however, by increasing the concentration of the salts within the gel. The results illustrating this statement are given in Table III, and plotted graphically in fig. 2. In these experiments the gels were made by heating the following solutions :—

2 c.c. 4-per-cent. sodium cholate + 0.5 c.c. 3N/10  $\text{CaCl}_2$  + 1.5 c.c. 2N/9 KCl.  
 " " " " " " 1.5 c.c. 3N/9 KCl.  
 " " " " " " 1.5 c.c. 4N/9 KCl.

The concentration of potassium chloride in these gels was, therefore, N/12, N/8, and N/6. The eroding solution used was that of sodium chloride.

The influence of certain non-electrolytes on gels was also investigated. Dextrose solutions have practically no erosive action on the gels, whether they contain added salts or not. On the other hand, the erosive action of salt solutions on gels containing dextrose is similar to that on gels containing

\* The curves are not reproduced in this paper.

salts, and the curves representing the action give indications of the same biphasic character. The action of sodium chloride solution on gels

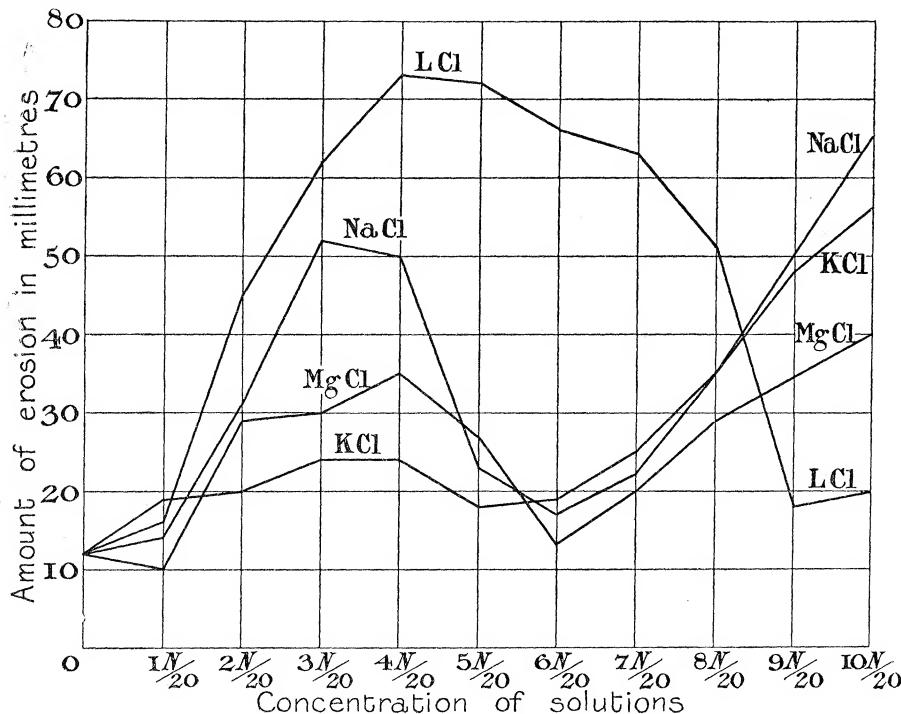


FIG. 2.

Table III.—Erosive Action (measured in mm.) of Sodium Chloride Solutions on Cholate Gel containing Varying Concentrations of Potassium Chloride.

Concen- tration of KCl in gel.	Concentration of NaCl solutions.									
	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
N/12 ...	16	16	27	17	14	16	28	44	52	59
N/8 .....	18	18	28	40	26	12	18	28	38	47
N/6 .....	19	24	47	56	44	20	14	20	30	37

Erosion in water 14.

containing dextrose is illustrated by Table IV. The gels were made from the following mixtures:—

2 c.c. 4-per-cent. sodium cholate + 0.5 c.c. 3 N/10 CaCl<sub>2</sub> + 1.5 c.c. N dextrose.

“ “ “ “ “ “ 1.5 c.c. 3N/9 “

The concentration of dextrose in the gels was, therefore, 3N/8 and N/8.

Table IV.—Erosion of Gels (measured in mm.) containing Dextrose by Sodium Chloride Solutions.

Concentration of dextrose in gel.	Concentration of sodium chloride.										
	0.	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.	9N/20.	10N/20.
3N/8 .....	13	14	18	37	49	49	51	69	57	41	40
N/8 .....	13	22	—	35	31	24	20	20	21	21	24

*Antagonistic Action of Calcium Chloride to Sodium Chloride.*

Experiments were undertaken to ascertain the amount of calcium chloride necessary to antagonise the erosive action of sodium chloride solutions of various concentrations. It will be observed from Table V, in which the results of these experiments are recorded, that the erosion produced in a gel (containing N/8 KCl) by a concentration of 3N/20 sodium chloride is about equal to that produced by the concentration 8N/20, when no calcium chloride is added to the eroding solutions. It was found that somewhat higher concentrations of calcium chloride were necessary to produce complete protective action against the 8N/20 solution than were required in the case of the 3N/20 solution of sodium chloride. Nevertheless, the ratio of calcium salt to sodium salt necessary to inhibit erosion within the "zone of instability" was larger than that which was required to produce complete antagonism in the region of the second phase of the erosion curve.

Table V.—Antagonistic Action of  $\text{CaCl}_2$  to various Concentrations of  $\text{NaCl}$ . Gel. 2 c.c. 4-per-cent. sodium cholate + 0.5 c.c. 3N/10  $\text{CaCl}_2$  + 1.5 c.c. N/3 KCl.

Concentration of $\text{NaCl}$ .	Amounts of erosion (in millimetres).				
	In absence of $\text{CaCl}_2$ .	In presence of N/124 $\text{CaCl}_2$ .	In presence of N/64 $\text{CaCl}_2$ .	In presence of N/32 $\text{CaCl}_2$ .	In presence of N/16 $\text{CaCl}_2$ .
2N/20 .....	18	6	5	3	0
3N/20 .....	37	6	4	3	0
4N/20 .....	44	7	4	2	0
5N/20 .....	21	6	4	3	0
6N/20 .....	14	7	5	2	0
7N/20 .....	17	12	4	3	0
8N/20 .....	35	22	5	4	0
9N/20 .....	39	26	8	4	0

Finally, the action of various sodium salts (organic or inorganic) on cholate gels was investigated. When the gel was made in the ordinary

manner without added salts, the diphasic erosion curves were not obtained, and, generally, the solutions with low surface tensions had a greater erosive action than those with higher tensions. When the gel contained an added salt, however, a diphasic curve was always obtained, but the breadth of the "zone of instability" and the amount of erosion within this zone varied from salt to salt. In the second phase of the curve the solutions of low surface tensions exerted the greatest erosive action. Of particular interest is the action of sodium lactate on the gel containing potassium chloride (N/8), which shows a very narrow "zone of stability," but a very active erosion within this zone. The results are given in Table VI.

Table VI.—Erosive Action of Sodium Lactate on Cholate Gels.

	Concentration of Lactate.							
	N/20.	2N/20.	3N/20.	4N/20.	5N/20.	6N/20.	7N/20.	8N/20.
Gel without added salt	11	15	40	>75	>75	>75	>75	>75
Gel containing N/8 KCl	17	>75	>75	21	33	>75	>75	>75

It will be noticed that the erosion is very large in the concentration 2N/20—3N/20, but small in that of 3N/20—4N/20.

The salt solutions in all these experiments were made by neutralising exactly to phenolphthalein 2N solutions of sodium hydroxide with the acid, and then diluting with water till the concentrations were normal.

#### THEORY OF THE ACTION OF SALTS ON GELS.

The results recorded indicate that the action of salts on a gel system is a complex one, and that the stability is in all probability the resultant of several different factors acting simultaneously. In the following pages an attempt is made to summarise these. Certain of the generalisations will refer more especially to the cholate gel, whereas others will apply to gel structures in general.

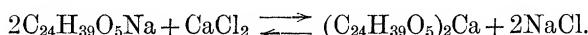
The phenomena accompanying the formation of the cholate gel have been described in a former paper,\* and these indicate that the gel itself consists of two phases, viz., a cholate-poor phase (designated hereafter as the "aqueous phase") and a cholate-rich phase (designated as the "cholate phase"), which during gel-formation gradually increases in bulk until its parts are more or less coterminous throughout the system. When this gel is introduced into a

\* 'Roy. Soc. Proc.,' B, vol. 87, p. 366 (1914).

salt solution a third phase is introduced, so that the total effect of erosive salt action must be studied in a triphasic system.

The cholate phase of the gel consists apparently of heavily hydrated aggregates of cholate salt which may vary (*a*) in composition (nature of cholate salt, degree of hydration, etc.), (*b*) in state of aggregation. It is assumed that it is upon these two factors the stability of the gel structure will mainly depend, and it remains to be considered how they may be affected by the salts in the triphasic system in which the erosion is studied. The possible actions of the salts are considered under six headings.

*I. The Influence of the Salts on the Cholate Component in the Cholate Phase.*—In the ordinary method of the formation of the gel, there is a reaction between calcium chloride and sodium cholate. The nature of this reaction is not, so far, clear. It is not an ordinary double decomposition, with the formation of calcium cholate as an insoluble gel-like substance. Such a method of gel-formation has been discovered in another system under investigation by one of the authors of this paper (S. B. S.), who has found that when calcium chloride is added to a solution of the sodium salt of plant pectin (which is an acid) a gel is produced immediately by precipitation. The cholate gel, on the other hand, forms only slowly at ordinary temperatures. It is not possible to state at the present what proportions of the cholate are present in the cholate phase as calcium and sodium salts. Probably the reaction between calcium chloride and sodium cholate may be represented in the form of a reversible reaction.



The addition of sodium chloride in excess, or of other chlorides, would, if this equation truly represents the facts, influence the composition of the cholates formed. Further work is necessary to determine the composition of the phases produced in the process of gel-formation.

*II. Influence of Salts on the Composition of the Two Phases of the Gel Resulting from the Establishment of Osmotic Equilibrium between these Phases.*—The cholate gel is an example of the special case in which the action of a non-diffusible ion (cholate) holds electrostatically a diffusible one (the metallic ion Ca, K, Na, etc.). The distribution of the components between the two gel phases to establish osmotic equilibrium would probably proceed in accordance with the hypothesis of Donnan, elaborated in the course of his investigations of membrane equilibria and potentials in the presence of non-dialysable electrolytes.\*

*III. Influence of the Salts on the Electric Charge on the Particles of the*

\* Donnan, 'Zeitsch. f. Elektrochemie,' vol. 17, p. 572 (1911).

*Cholate Phase of the Gel.*—It is a well-established fact that the state of aggregation of a colloid is subject to the influence of the charge it carries, and that this charge can be affected by the presence of salt ions. The action of these is a function of their nature and valency, the multivalent ions exerting the greatest amount of influence. The suspensoid (lyophobe) colloids are more especially sensitive to this particular action of salt ions, but it has been shown by Mines\* that the hydrated (emulsoid or lyophil) colloids are by no means insensitive. Preliminary experiments indicate that the cholate gel is affected by the valency of the anions, but experiments dealing with this possible factor are not yet complete.

IV. *The Influence of Salts on the Distribution of Water between the Two Phases of the Gel.*—As an extreme instance of the action of salts on the distribution of water between two phases, the salt precipitation of proteins may be quoted. This action is ascribed to the withdrawal of water from the protein phase† and the efficiency of salts in producing such an action is, most probably, a function of their state of hydration in solution. It is conceivable that in a two-phased gel system salts may exert an analogous action (by withdrawing water from the phase containing the larger amount of the substance to which gel formation is due, in the special case under consideration the cholate phase), although the effects may not be so readily demonstrable as in the case of proteins and only be revealed by a detailed analysis of the separate phases.

V. *Influence of Salts due to their Effect on the Surface Tension of Water.*—It has been shown that the greater the lowering of the surface tension of water produced by dissolving a given salt, the greater is the disaggregating action of the solution.‡ It has already been pointed out that the rate of formation of the cholate gel by different calcium salts is partly a function of the surface tensions of their solutions; the lower the surface tension of the calcium salt solution, the more slowly does gel-formation take place. In the case of certain calcium salts, gel-formation can be inhibited entirely when the concentration of the calcium salts is sufficiently great.§ Some experiments on the reversal of the gel by salts, which are not given in detail in this paper, indicate that, at any rate in the second phase of the erosion curve, the action of the salts which markedly lower the surface tension of water is on the whole greater than those which produce aqueous solutions of higher

\* 'Kolloidchemische Beihefte,' vol. 3, p. 191 (1911-12).

† See especially Harriette Chick and C. J. Martin, 'Biochem. Jour.', vol. 7, p. 380 (1913).

‡ For examples see Schryver, 'Roy. Soc. Proc.,' B, vol. 83, p. 96 (1910).

§ Schryver, 'Roy. Soc. Proc.,' B, vol. 87, p. 366 (1914).

surface tension. The viscosity of salt solutions also probably plays some part in that disaggregating action of salts which is connected with their effect on surface tension of water, owing to their forming a kind of diffusion layer round the colloid phase. This action has been already discussed in some detail in an earlier paper.\*

VI. *Influence of Salts due to Differences in Diffusion Rates.*—Salts will diffuse from one phase to another at different speeds, and the rate of interchange between the phases will be a function of this factor, which is such an obvious one that it needs no detailed discussion. It is possible that salts also exert other actions which have not been enumerated here.

From the foregoing remarks it would appear probable that the action of salts in a gel system is very complex and that it is hardly possible to investigate each single factor in a system such as that of the cholate gel; the results recorded in this paper must, therefore, be regarded as somewhat empirical in character. In this and in the earlier papers of this series on gel-formation, attention has been repeatedly drawn to the analogies between the physical characters of the cholate gel and certain vital activities of the cell, and if, as has been suggested, the protoplasm and the cell membrane contain a gel structure like that of the cholates, it is obvious that the biological action of salts must be a very complex one, which cannot be ascribed to the action of any one single factor acting separately. It is hoped that by a continuation of this work more light may be thrown on certain important physiological problems, especially those connected with the permeabilities of cells and their functional changes. Attempts have already been made to trace further analogies between the action of salts in producing such changes and their erosive action on cholate gels.

#### SUMMARY.

1. The erosive action of chloride solutions on a cholate gel containing added chlorides has been investigated. If the concentrations of the eroding solutions are plotted as abscissæ and the amounts of erosion as ordinates, a curve is obtained which is of diphasic character. The amount of erosion increases with increasing concentration to a maximum, and then, with further increase in the concentration of the eroding solution, it diminishes to a minimum; on increasing the concentration beyond this latter point, the amount of erosion increases continuously. The portion of the curve between the two minimal points is designated the "zone of instability" of the gel.

2. The breadth of this zone and the amount of erosion within it is a

\* Schryver, 'Roy. Soc. Proc.,' B, vol. 83, p. 96 (1910). See also Traube, 'Internationale Zeitsch. f. physikalisch-chemische Biologie,' vol. 1, p. 275 (1914).

function of the amount of salt added to the gel; the larger this amount, the broader the "zone of instability" and the greater the amount of erosion within it.

3. An erosion curve of similar form is obtained when a non-electrolyte (dextrose) is added to the gel. The sugar is, however, without action when present in the eroding fluid, whether the gel contains added salts or not.

4. Quantitatively, the chloride solutions in their erosive action differ considerably from one another; the differences between their action is less marked when present in the gel. The maximal effect, both as regards the breadth of the "zone of instability" and the amount of erosion within this zone, is produced by lithium chloride. The order of action of the salts investigated is  $\text{LiCl} > \text{NaCl}$  (generally)  $> \text{MgCl}_2 > \text{KCl}$ . This is the order of their action in increasing the permeability of certain vegetable cells.

5. Relatively more calcium chloride is necessary to "antagonise" the erosive action of salts within the "zone of instability" than is necessary to antagonise the same amount of erosive action produced by higher concentrations (in the second phase of the erosion curve).

6. The erosive action of sodium salts other than chloride on the gel containing added chlorides was also investigated, and an erosion curve of diphasic character was always obtained. The form differed for each salt, both as regards the breadth of the "zone of instability" and the amount of erosion. Specially remarkable was the erosion curve produced by sodium lactate on a gel containing potassium chloride. The erosion curve in this case showed a very narrow "zone of instability," with an exceptionally large amount of erosion within the zone.

7. The general action of salts on a gel system has been discussed, and it is pointed out that it is probably the resultant of several physico-chemical factors acting simultaneously. Attention is also called to the probable complexity of the biological action of salts.

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